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INTERNATIONAL STUDY ON *ARTEMIA*[1]. XXV. FACTORS DETERMINING THE NUTRITIONAL EFFECTIVENESS OF *ARTEMIA*: THE RELATIVE IMPACT OF CHLORINATED HYDROCARBONS AND ESSENTIAL FATTY ACIDS IN SAN FRANCISCO BAY AND SAN PABLO BAY *ARTEMIA*[2]

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Abstract: Different *Artemia* cyst samples harvested from the San Francisco and the San Pablo Bay regions (California, U.S.A.), and suspect because of their poor nutritional performance in fish and crustacean farming, have been analysed for their chlorinated hydrocarbon and fatty acid content. These results have been correlated with survival, growth, and biomass production of larvae of the marine crustacean *Mysidopsis bahia* Molenock fed those different *Artemia* in a standard culture test.

Differences in chlorinated hydrocarbon content do not correlate with differences in mysid culture performance. Fatty acid profiles reveal three groups of *Artemia* batches with high, intermediate, and low levels of the highly unsaturated fatty acid 20:5ω3. The production yield of the mysid larvae could be correlated with the relative level of the 20:5ω3 fatty acid in the *Artemia nauplii*.

Key words: *Artemia*; *Mysidopsis bahia*; nutrition; chlorinated hydrocarbons; fatty acids

INTRODUCTION

Artemia nauplii are used as a convenient and nutritious food source for larval rearing of many fish and crustacean species. Several publications, however, report significant variations in nutritional effectiveness of *Artemia* nauplii from different geographical origins (Bookhout & Costlow, 1970; Johns *et al.*, 1980, 1981; Watanabe *et al.*, 1980; Beck & Bengtson, 1982; Léger & Sorgeloos, 1984). Although various explanations for poor culture success with specific *Artemia* sources have been proposed by different authors to date no conclusive answer has been put forward. The most recurring

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suggestions, when size of nauplii does not interfere (Beck & Bengtson, 1982), claim pesticide contamination (Bookhout & Costlow, 1970; Helfrich, 1973; Olney *et al.*, 1980), lack of essential fatty acids (Watanabe *et al.*, 1978; Fujita *et al.*, 1980; Schauer *et al.*, 1980; Léger *et al.*, 1984), or an interaction of the above (Klein-MacPhee *et al.*, 1980; Olney *et al.*, 1980; Schauer *et al.*, 1980).

During the period 1978–1981 we received information from several commercial and experimental farmers of fish, crab, shrimp and prawn about increased larval mortalities which appeared to be attributable to the use of specific batches of *Artemia* cysts from the San Francisco Bay (California, U.S.A.) area. In order to ascertain the relative impact of contamination (i.e. chlorinated hydrocarbons) and of essential fatty acids (i.e. highly unsaturated long-chain fatty acids) a series of chemical analyses and culture tests have been done with these suspected as well as other batches of San Francisco Bay *Artemia*. Bioassays were run with the marine mysid crustacean *Mysidopsis bahia* Molenock which can be fed throughout its life with *Artemia* nauplii.

Materials and Methods

Artemia samples

Cyst batches used in this study were collected from salt ponds in the San Francisco Bay area (batches SFB I through SFB XIV, all harvested in the period 1976–1980) and from salt ponds in the more northern San Pablo Bay (California, U.S.A.) area (batch SPB). Reference *Artemia* cysts (RAC; Sorgeloos, 1980), selected for their demonstrated high quality as food for various fish and crustacean predators (Seidel *et al.*, 1982) have been used as a control in all tests.

Artemia cysts were incubated in filtered artificial sea water (formula of Dietrich & Kalle, in Kinne, 1970; S 30‰, 25 °C, 0.2 μm filtration) under continuous illumination (2000 lux) and aeration. Freshly hatched Instar I nauplii were harvested, separated from hatching debris and thoroughly rinsed after T_{90} h of cyst incubation (T_{90} = incubation time for 90% hatch; Vanhaecke & Sorgeloos, 1982).

Chlorinated hydrocarbon analysis

The analytical procedure used was similar to the one reported by Olney *et al.* (1980) for the chlorinated hydrocarbon content of various geographical selections of *Artemia*. The cysts were either extracted directly or hatched, collected, and freeze-dried prior to extraction. The samples were weighed and then homogenized in a Polytron Homogenizer and extracted with light petroleum, concentrated by a Kuderna–Danish evaporative concentrator and chromatographed on alumina. Two fractions were collected. The first, containing PCBs and the majority of the pesticides was further fractionated on silicic acid, primarily to separate the PCBs from the other components. The second fraction from alumina was analysed for dieldrin and endrin before and after treatments with sulphuric acid and alcoholic potassium hydroxide.

Individual column fractions were analysed by dual column electron capture gas chromatography (Tracor MT-220 gas chromatograph, Ni-63 detector, 180 × 0.4 cm glass columns packed with 1.5% OV-17/1.95% QF-1 or 4% SE-30/6% QF-1 on 100/120 mesh supelcon AW-DCMS). Peaks were identified by retention times and quantified using peak heights as compared with standards. The residues reported have been corrected for reagent blanks and the nauplii values represent duplicate analyses of 2- to 6-g samples (cf. Olney *et al.*, 1980).

FATTY ACID ANALYSIS

Fatty acid profiles were determined on freshly hatched Instar I *Artemia* nauplii by capillary gas chromatography. Nauplii were homogenized with an ultrasonic homogenizer (Sonifier B12). Lipid extraction, saponification and esterification was done according to the procedure described by Schauer & Simpson (1978). Fatty acid methyl esters were injected on a capillary column (25 m fused silica, I.D.: 0.32 mm, liquid phase: SILAR 10C, film thickness: 0.3 μm) installed in a Carlo Erba Fractovap 2330 gas chromatograph. Operating conditions were as follows: solid injector, carrier gas: hydrogen, flow rate: 1.9 ml $\cdot$ min^{-1}, F.I.D., oven temperature programme: 154 to 200 °C at 2 °C $\cdot$ min^{-1}. Peak identification and quantification was done with a calibrated plotter-integrator (H.P. 3390A) and reference standards.

MYSIDOPSIS BIOASSAY TEST

A homogenous population of freshly emerged juveniles of *Mysidopsis bahia* was obtained with the mysid incubator-separator as described by Léger & Sorgeloos (1982). The experiment consisted of 12 treatments in each of which the mysids were fed a different batch of *Artemia* nauplii. Each treatment contained nine replicate 1-litre bowls filled with 500 ml filtered (0.2 μm) artificial sea water (S 30‰, formula of Dietrich & Kalle, in Kinne 1970) and stocked with 10 experimental animals each. The experiment was conducted at 25.0 $\pm$ 0.1 °C at a 12 : 12 photoperiod.

Mysids were daily fed ad libitum with freshly hatched Instar I *Artemia* nauplii during a 12-day period. Dead mysids and excess *Artemia* were removed daily and the culture water was completely changed after 4 and 8 days. At the end of the experiment, final survival, total length of live animals (dissection microscope equipped with drawing mirror) and individual dry weight (24 h desiccation at 60 °C; analytical balance 1 μg accuracy) were determined. The product of number of survivors and mean individual dry weight was also calculated and considered as the estimated biomass production.

Data were treated statistically in a one-way analysis of variance (Snedecor & Cochran, 1967). Duncan's multiple range test was used to determine significant differences between means (Goodnight, 1979). Linear and stepwise regressions were calculated to determine correlations between bioassay results and contamination with chlorinated hydrocarbons, on one hand, and polyunsaturated fatty acids, on the other hand.

RESULTS

CHLORINATED HYDROCARBONS

Data on the chlorinated hydrocarbon content of dry cysts and freeze-dried nauplii are presented in Tables I and II, respectively. Considerable differences in concentration among batches of dry cysts can be noticed for *t*-nonachlor (nd–36 $ng \cdot g^{-1}$), dieldrin (nd–10.7 $ng \cdot g^{-1}$), pp-DDE (8–61 $ng \cdot g^{-1}$) pp-DDD (nd–46 $ng \cdot g^{-1}$), op-DDT (nd–13 $ng \cdot g^{-1}$), pp-DDT (nd–10 $ng \cdot g^{-1}$), PCB 1016 (6–144 $ng \cdot g^{-1}$) and PCB 1254/1260 (1–124 $ng \cdot g^{-1}$). On the other hand, chlorinated hydrocarbon profiles in freeze-dried *Artemia* nauplii (Table II) reflect those of the cysts, but concentrations are often twice or more as high.

FATTY ACID PROFILE

The fatty acid profile of freshly hatched *Artemia* nauplii from Reference *Artemia* Cysts and from the San Pablo Bay and the San Francisco Bay batches are given in Table III. Clear differences can be noticed especially for the fatty acids 18 : 3ω3 and 20 : 5ω3.

Three groups of SFB cyst batches can be distinguished:

(1) relatively high in 16 : 1ω7, ω9 and 20 : 5ω3, relatively low in 18 : 2ω6, 18 : 3ω3 and 18 : 4ω3 : SFB I and II;

(2) relatively low in 16 : 1ω7, ω9 and 20 : 5ω3, relatively high in 18 : 2ω6, 18 : 3ω3 and 18 : 4ω3: SFB III, IV, V, VI, VII, VIII, IX and X;

(3) intermediate values for 16 : 1ω7, ω9, 20 : 5ω3, 18 : 3ω3 and 18 : 4ω3: SFB XI, XII, XIII and XIV.

RAC can be ranged under Group 1, except for a high level of 18 : 2ω6, while SPB is to be ranged under Group 2.

MYSIDOPSIS BIOASSAY

Data on survival, growth, and biomass production of *Mysidopsis bahia* juveniles fed freshly hatched *Artemia* nauplii from RAC, San Pablo Bay and 10 different San Francisco Bay batches are given in Table IV. Significant differences ($P = 0.05$) are noticed for survival, individual dry weight and length of the test animals after culturing for 12 days. It also appears that low survival does not exclude good growth (cf. SFB XII). Differences in growth seem to be more pronounced in terms of individual dry weight than in terms of individual length. The estimated biomass production data clearly illustrate differences in culture success as a function of the batch of *Artemia* cysts used as food source for *Mysidopsis bahia* juveniles.

In an attempt to correlate survival, growth, and biomass production of mysids with chlorinated hydrocarbon content (individual CHCs as well as sum of CHCs) and levels of polyunsaturated fatty acids (18 : 2ω6, 18 : 3ω3, 18 : 4ω3, 20 : 4ω3, and 20 : 5ω3), only mysid biomass and 20 : 5ω3 content of *Artemia* had a high degree of positive correlation:

TABLE I

Chlorinated hydrocarbons in Reference *Artemia* Cysts (RAC), San Pablo Bay *Artemia* (SPB) and different San Francisco Bay *Artemia* cyst batches (SFB I–XIV): results are expressed in ng per g dry wt; *, based on one analysis only; +, data calculated from Seidel *et al.*, 1982; nd, not detected.

	SFB														SPB	RAC+
	I	II	XI	XII	XIII	XIV	III	IV	V	VI	VII	VIII	IX	X		
HCB	0.7	0.7	0.8	0.6	0.8	0.6	1.2	1.0	1.0	1.1	0.6	1.3	0.8	0.8	1.0	1.6
α-BHC	0.9	2.7	2.3	1.7	0.8	4.0	1.5	2.8	2.4	0.7	1.6	2.6	1.6	2.6	3.1	1.1
γ-BHC	0.6	1.7	1.9	4.5	1.9	3.2	2.8	3.0	1.9	3.0	1.8	2.3	2.5	3.6	3.2	nd
o-chlordane	nd	5	3	4	3	2	2	5	4	3	2	17	4	3	6	0.5
t-chlordane	nd	10	6	7	8	6	9	9	9	8	8	7	10	8	14	nd
t-nonachlor	nd	24	16	13	12	13	23	17	22	13	20	13	19	17	36	nd
Σ-chlordanes	nd	39	25	24	23	21	34	31	35	24	30	37	33	28	56	0.5
dieldrin	nd	4.8	5.3	2.5	3.9	10.7	4.5	10.4	3.0	2.9	2.8	nd	2.2	7.3	5.6	nd
pp-DDE	11*	15	23	28	30	16	61	22	18	15	17	34	17	21	50	8
pp-DDD	nd	43	23	31	15	18	30	38	24	42	19	46	20	37	37	2
op-DDT	nd	4	2	7	1	5	3	3	3	8	2	13	1	4	4	nd
pp-DDT	nd	5	6	6	5	4	5	6	4	5	4	10	2	8	10	2
Σ DDTs	11	67	54	72	51	43	99	69	49	70	42	103	40	70	101	12
PCB 1016	32*	27	42	60	61	30	144	46	21	38	23	73	20	46	37	6
PCB 1254/1260	10*	36	44	56	56	36	124	44	35	36	46	112	36	44	68	1
Σ PCBs	42	63	86	116	117	66	268	90	56	74	69	185	56	90	105	7

TABLE II

Chlorinated hydrocarbons in freeze-dried San Pablo Bay *Artemia* (SPB) and different batches of San Francisco Bay *Artemia* (SFB) nauplii: results are expressed in ng per g dry wt.

	SFB				SPB
	II	VII	V	IX	
HCB	1.5	1.0	1.3	1.2	1.6
α-BHC	5.0	4.0	3.5	3.0	4.5
γ-BHC	4.5	4.0	3.8	4.0	6.0
o-chlordane	6	5	6	6	8
t-chlordane	24	15	20	19	34
t-nonachlor	56	37	53	52	71
Σ-chlordanes	86	57	79	77	113
dieldrin	12.5	8.5	10.0	9.0	21.5
pp-DDE	21	23	27	24	69
pp-DDD	77	43	53	43	53
op-DDT	11	6	4	2	11
pp-DDT	8	6	7	4	18
Σ DDTs	117	78	91	73	151
PCB 1016	33	48	31	34	50
PCB 1254/1260	74	89	68	60	91
Σ PCBs	107	137	99	94	141

$Y = 1.95\ (X1) + 13.3$, $r = 0.90$, Y = mysid biomass, $X1$ = 20 : 5ω3 content in *Artemia*

A negative correlation was found between mysid biomass and 18 : 2ω6, 18 : 3ω3 and 18 : 4ω3 content in *Artemia*

$Y = -0.29\ (X2) + 13.4$, $r = -0.90$, Y = mysid biomass, $X2$ = 18 : 2ω6 content in *Artemia*

$Y = -0.57\ (X3) + 31.7$, $r = -0.84$, $X3$ = 18 : 3ω3 content in *Artemia*

$Y = -0.24\ (X4) + 8.5$, $r = -0.83$, $X4$ = 18 : 4ω3 content in *Artemia*

Chlorinated hydrocarbon content did not correlate well with results of the mysid culture test:

e.g. $Y = -0.03\ (X5) + 24.9$, $r = 0.38$, Y = mysid biomass, $X5$ = sum of CHCs in *Artemia*

TABLE III

Fatty acid profile of freshly hatched Reference *Artemia* cysts (RAC), San Pablo Bay *Artemia* (SPB) and different batches of San Francisco Bay *Artemia* (SFB) nauplii: results are expressed in per cent fatty acid methyl ester of total fatty acid methyl esters; F.A.M.E., fatty acid methyl ester; –, not detected; n.i.p., non-identified peaks.

F.A.M.E.	RAC	SFB														SPB
		I	II	XI	XII	XIII	XIV	III	IV	VI	V	VII	VIII	IX	X	
14:0	1.6	1.7	1.4	1.2	0.8	1.1	0.9	1.0	0.9	0.8	0.6	0.8	0.8	0.6	1.3	0.5
14:1	1.2	1.0	0.5	1.0	0.9	0.8	1.0	1.3	1.1	1.2	1.2	1.0	1.5	1.1	0.9	1.9
14:2	0.3	–	0.2	0.2	trace	–	0.2	0.3	0.3	0.2	0.2	0.1	0.3	0.2	0.2	–
15:0	0.8	0.4	0.3	0.6	0.4	0.4	0.4	0.5	0.4	0.4	0.4	0.4	0.5	0.4	0.4	trace
15:1	0.7	0.2	0.2	0.5	0.4	0.3	0.5	0.7	0.6	0.6	0.5	0.4	0.7	0.5	0.5	0.6
16:0	12.7	12.5	13.0	11.6	11.5	12.2	5.9	11.3	11.3	10.0	9.5	11.7	9.0	10.2	10.8	9.3
16:1ω9	1.2	20.9	0.4	0.7	0.6	0.5	0.6	0.7	0.6	0.7	0.6	0.5	0.6	0.6	0.5	0.8
16:1ω7	13.3		19.6	6.6	7.1	6.6	6.7	5.4	5.4	4.3	4.3	3.9	4.0	3.7	3.7	4.0
16:2	1.2	0.7	0.3	0.5	0.5	0.3	0.4	0.6	0.5	0.4	0.5	0.4	0.5	0.5	0.4	6.7
16:3	3.4	0.7	1.1	1.9	1.3	1.4	1.7	1.6	1.6	1.6	1.5	1.4	1.8	1.4	1.5	0.9
17:0	1.8	–	0.3	0.9	0.5	0.5	0.4	0.7	0.7	0.5	0.6	0.5	0.5	0.6	0.6	–
18:0	4.2	3.0	3.0	3.5	3.7	3.0	1.6	3.2	3.4	2.2	3.0	2.9	2.0	3.3	3.9	3.6
18:1ω9	19.2	34.9	34.7	28.5	28.2	30.8	32.2	27.0	26.8	32.0	24.7	27.3	27.4	24.4	27.9	26.3
18:1ω7	12.6						0.6	0.6		0.7	3.8		0.9	3.8		0.7
18:2ω6	9.2	3.0	4.7	6.9	8.2	7.5	8.5	7.9	8.3	9.2	8.7	10.0	9.1	9.7	8.1	9.9
18:3ω6	0.4	2.0	0.2	0.8	0.8	0.6	0.9	1.0	0.8	0.9	0.9	0.7	1.0	0.8	0.6	trace
18:3ω3	1.4	5.9	7.5	18.7	20.9	22.2	25.6	23.6	23.3	26.3	27.2	28.0	27.6	26.3	27.7	31.0
18:4ω3	0.4	1.3	1.4	3.7	3.8	3.2	3.9	4.6	4.3	4.4	4.8	4.1	4.7	5.1	5.4	7.0
20:0	0.1	0.2	0.1	0.1	trace	trace	trace	0.1	0.3	0.1	0.1	0.1	0.1	0.1	0.1	–
20:1	0.7	1.2	0.9	1.4	1.5	0.5	0.8	1.5	1.1	1.6	0.5	0.2	1.7	1.4	0.6	–
20:2ω6	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.2	0.3		0.3
20:3ω6	0.3	0.4	0.1	0.4	0.1	0.2	–	0.3	0.3	0.1	0.1	0.1	0.1	0.1	0.2	0.1
20:3ω3	0.1				0.2	–	0.2		–	0.1	0.2	0.2	0.1	0.1	0.1	
20:4ω6	0.1	–	0.1	0.2	0.1	0.1	0.1	–	0.1	0.2	–	–	0.2	0.2	0.1	0.5
20:4ω3	4.7	2.1	1.8	1.2	1.3	1.1	1.8	0.5	0.5	1.4	0.9	0.9	0.7	0.5	0.5	
20:5ω3	7.4	8.8	8.2	4.7	3.6	3.4	2.8	1.8	1.7	1.6	1.5	1.4	1.2	0.7	0.6	0.2
22:1	0.2	0.4	–	0.5	0.7	0.4	0.5	0.7	0.7	0.5	0.8	0.7	0.7	0.8	0.9	–
22:3ω3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
22:4ω6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
22:4ω3	–	–	trace	–	–	–	–	–	0.2	–	–	–	0.1	0.1	–	–
22:5ω6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
22:5ω3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
22:6ω3	–	0.6	trace	–	–	–	–	–	0.1	–	–	–	–	–	–	–
24:0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
24:1	–	–	–	–	–	–	–	–	–	–	–	–	–	0.2	–	–
n.i.p.	1.0	–	–	3.5	2.7	2.6	1.6	2.9	4.7	0.8	1.7	2	2.0	2.3	2.5	–

TABLE IV

Data on survival, individual dry wt, individual length, and biomass of *Mysidopsis bahia* fed freshly hatched *Artemia* nauplii from Reference *Artemia* Cysts (RAC), San Pablo Bay (SPB) and different San Francisco Bay batches (I, II, III, V, VII, IX, X, XI, XII, XIII) in a standard culture test: data on selected fatty acids and chlorinated hydrocarbon contaminants are also given; a, b, c, means with different superscript are significantly ($P = 0.05$) different.

Artemia batch	I	II	RAC	XI	XIII	XII	III	X	V	VII	SPB	IX
Mysid test												
Survival (%)	93.3^{a}	93.3^{a}	91.6^{a}	90.1^{a}	84.0^{b}	52.0^{c}	78.5^{b}	74.8^{b}	60.0^{c}	56.8^{c}	53.2^{c}	40.7^{c}
σ	5.8	9.5	10.9	10.8	14.7	8.1	11.3	4.4	10.0	8.9	10.2	17.4
Individual dry wt	363^{a}	319^{bc}	327^{b}	337^{b}	309^{bc}	382^{a}	254^{d}	258^{d}	310^{bc}	310^{bc}	268^{cd}	234^{d}
(μg) σ	47	44	44	33	39	45	25	29	54	31	32	82
Individual length	5470^{a}	4467^{bc}	4697^{b}	4450^{bc}	4391^{cd}	4462^{bc}	4272^{cd}	4128^{d}	4103^{d}	4449^{bc}	4072^{d}	4113^{d}
(μm) σ	471	185	126	146	104	237	128	219	286	207	81	324
Biomass (mg)	30.5	26.8	27.0	27.3	23.4	17.9	17.9	17.4	16.7	15.8	12.8	8.6
Fatty acids (area %)												
18:3ω3	5.9	7.5	1.4	18.7	22.2	20.9	23.6	27.7	27.2	28.0	31.0	26.3
20:5ω3	8.8	8.2	7.4	4.7	3.4	3.6	1.8	0.6	1.5	1.4	0.2	0.7
Chlorinated hydrocarbons ($ng \cdot g^{-1}$)												
Chlordanes	nd	39.0	0.5	25.0	23.0	24.0	34.0	28.0	35.0	30.0	56.0	33.0
Dieldrin	nd	4.8	nd	5.3	3.9	2.5	4.5	7.3	3.0	2.8	5.6	2.2
PCB 1254/1260	10.4	36.0	1.1	44.0	56.0	56.0	124.0	44.0	35.0	46.0	68.0	36.0
Σ C.H.C.	55.5	178.9	21.4	175.3	198.4	221.3	411.0	202.3	148.3	147.8	247.9	136.1

This low degree of correlation was confirmed by a stepwise multiple regression of the form: $Y = a(X1) + b(X5) + c$ which yielded the following equation:

$Y = -2.07\ (X1) + 0.0074\ (X5) + 11.6$, Y = mysid biomass, $X1 = 20:5\omega3$ content in *Artemia*, $X5$ = sum of CHCs in *Artemia*

This indicates that only $X1$ ($20:5\omega3$ content in *Artemia*) is significantly contributing to the equation, explaining 99% of the sum of squares for the regression.

Discussion

Comparison of the chlorinated hydrocarbon content of *Artemia* cysts and of freeze-dried nauplii shows that naupliar profile reflects that of the cysts. Concentrations, however, are higher in the nauplii – often twice or more – indicating that chlorinated hydrocarbons are concentrated in the embryo. Pesticide diffusion from the environment into the embryo is excluded since these molecules are too big to pass through the outer cuticular membrane (Morris & Afzelius, 1967). Therefore, contamination of oviparous *Artemia* nauplii can only originate from bio-accumulation by parental animals.

From the results of this study it also appears that the nutritional effectiveness for *Mysidopsis bahia* of *Artemia* nauplii from San Pablo Bay and different batches of San Francisco Bay origin cannot be correlated with contamination levels of individual or total chlorinated hydrocarbons in those *Artemia* nauplii. This disproves the supposition of Olney *et al.* (1980) that dieldrin, chlordane and high molecular PCBs would appear to be the most likely suspects for the poor nutritional value of San Pablo Bay *Artemia*. Epifanio (1972) also found that dieldrin levels in brine shrimp of 150 $ng \cdot g^{-1}$ did not affect larval survival of the crab *Leptodius floridanus*. Further confirmation of the present findings is found in Johns *et al.* (1981) who showed that artificial contamination of quasi pesticide-free *Artemia* nauplii from Brazil with chlordane (up to 93 $ng \cdot g^{-1}$) and dieldrin (up to 170 $ng \cdot g^{-1}$) had no effect on growth or survival of *Rhithropanopeus harrissii* larvae. McLean (1980) fed *Artemia* contaminated with the similar levels of chlordane and dieldrin as applied by Johns *et al.* (1981) to winter flounder larvae, *Pseudopleuronectes americanus*. Although no mortalities were noted size differences did correlate with the contamination level. It should be noticed, however, that especially dieldrin levels were much higher than these naturally encountered in the *Artemia* batches used in this study.

Fatty acid profile in the *Artemia* nauplii is better correlated with the results of the bioassay test: i.e. the relative concentrations of $20:5\omega3$ is positively correlated, whereas the relative concentrations of $18:2\omega6$, $18:3\omega3$, and $18:4\omega3$ are negatively correlated with mysid biomass production. Indeed, fatty acid profile more than observed chlorinated hydrocarbon contamination, seems to play a determining rôle in the nutritional effectiveness of *Artemia* nauplii. Since highly unsaturated fatty acids are essential for

marine predators (Watanabe *et al.*, 1978), the presence of sufficient amounts of 20 : 5 ω3 must be one of the most important factors in determining the food value of San Francisco Bay *Artemia*. This is confirmed by the results of culture tests using *Artemia* from different strains as food for red seabream *Pagrus major* (Fujita *et al.*, 1980; Watanabe *et al.*, 1982), blue crab *Rithropanopeus harrissii* (Johns *et al.*, 1980), winter flounder *Pseudopleuronectes americanus* (Klein-MacPhee *et al.*, 1980), Atlantic silverside *Menidia menidia* (Beck *et al.*, 1980), and the blue shrimp *Penaeus stylirostris* (Léger *et al.*, 1984).

We have recently provided extra proof of the critical role of 20 : 5 ω3 in the nutritional effectiveness of *Artemia* for *Mysidopsis bahia* and *Penaeus stylirostris* (Léger *et al.*, 1984) by changing the fatty acid pattern in SPB *Artemia* (as in the work of Fujita *et al.*, 1980), through feeding the *Artemia* nauplii with 20 : 5 ω3-enriched and 20 : 5 ω3-lacking diets.

The fact that 18-carbon polyunsaturated fatty acids (18 : 2 ω6, 18 : 3 ω3, and 18 : 4 ω3) have a high degree of negative correlation with mysid biomass production does not mean that they exercise a negative nutritional effect. This connection of high, respectively, low levels of 20 : 5 ω3 with low, respectively, high levels of 18-carbon PUFAs in *Artemia* is a reflection of the same connection of those fatty acids in the *Artemia* diet. Indeed, Hinchcliffe & Riley (1972) and Vos *et al.* (1984) have shown that the fatty acid profile of *Artemia* is primarily determined by the fatty acid profile of the food (i.e. algae) ingested by the parent animals. This means that the nutritional effectiveness of *Artemia* is to a large extent determined by the nutritional quality of the food available where and when the cysts are produced.

A possible synergetic interactive effect of high levels of 18 : 3 ω3 and the presence of chlorinated hydrocarbons in *Artemia* as suggested by Schauer *et al.* (1980) and Olney *et al.* (1980) is not rejected or confirmed by this study. For this one needs a contamination-free 18 : 3 ω3-rich *Artemia* batch, which so far has not yet been identified. We conclude that the variable effect of feeding specific batches of *Artemia* is related to fatty acid composition, especially 20 : 5 ω3 content. It is not due primarily to levels of chlorinated hydrocarbons in these cysts.

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